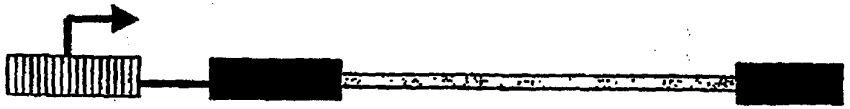
Figure 1 : Design for RNAi expression



Indicates the start of transcription

Intervening nucleotide sequence(s) of variable/flexible length

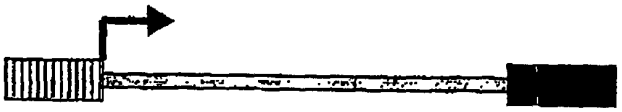
Stretches of nucleotides comprising RNAi, sense and antisense RNAi sequence(s) with or without nucleotide sequence(s) between the sense and antisense RNAi is described. Additional nucleotides may be present 5' and or 3' of the sense and or antise nse RNAi nucleotides. One or more additional elements to cleave the target nucleotides for example, cis-acting ribozyme(s) may be present between the sense and antisense RNAi nucleotides.

One or more elements to cleave the target nucleotides for example, cis-acting ribozyme(s)

Tissue specific and or cell specific and or general and or inducible promoter(s) and or control sequences e.g. enhancers

Expression of this cassette may be undertaken via inter alia a viral or non-viral vector, an artificial chomosome and or a stretch of DNA and or RNA

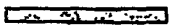
Figure 2 : Design for RNAi expression



Indicates the start of transcription. RNAi is transcribed close to the strart site for transcription

Optional inclusion of none, one or more elements to cleave the target nucleotides for example, cis-acting ribozyme(s) to cleave off for examle the polyA tail

Tissue specific and or cell specific and or general and or inducible promoter(s) and or control sequences e.g. enhancers



Stretches of nucleotides comprising RNAi, sense and antisense RNAi sequence(s) with or without nucleotide sequence(s) between the sense and antisense RNAi is described. Additional nucleotides may be present 5' and or 3' of the sense and or antise nse RNAi nucleotides. One or more additional elements to cleave the target nucleotides for example, cis-acting ribozyme(s) may be present between the sense and antisense RNAi nucleotides.

Number	Gene	Ref	5' Sequences driving tissue specific expression
1	Rhodopsin, RHO gene	Gouras et al. Vis Neurosci 11: 1227 1994	Photoreceptor specificty can beachieved using vaious sequences 5' of the TSS of the rhdopsin promoter (from –222 to +70 and –2174 to +70). Expression in both rod and cone photoreceptor cells is observed using these 5' sequences
2	Alpha 1 type I collagen	Kalajuzic et al. Bone 31: 654 2002	Expression in osteoblasts can be achieved using, for example, 3.6 kb of the rat type collagen 1A1 promoter
3	Oligodendrocyte myelin glycoprotein	Solly et al. Neurochem 68: 1705 1997	657 bases of the 5' murine MOG can drive gene expression of a gene in an oligodendroglial cell line
4	Cone arrestin (CAR)	Zhu et al. Febs letters 524: 116 2002	215 bases 5' fragment of the CAR gene can drive expression of a gen in retinoblastoma cells
5	Albumin	Postic et al. J Biol Chem 274: 305 1999	2.3 kilobases 5' of the albumin gene can drive expression of a gene in hepatocytes
6	Neuron specific enolase	Kowalski et al. Diabetes 30: 425 2001	1.8 kilobases of the neuron specific enolase drives gene expression in neurons
7	Human GnRH gene	Wolfe et al. Mol Endo 16: 435	Expression in neurons can be achieved using 992 bases of the human GnRH gene promoter
8	alpha-actin promoter	Frauli et al. Med Sci Monit 9: BR78 2003	Human skeletal alpha-actin promoter (between –432 and +239) and beta-enolase enhancer (+504 and +637) can direct gene expression in skeletal muscle
9	Smooth muscle myosin heavy chain promoter	Franz et al. Cariovasc Res 43: 1040 1999	2.3 kilobsaes of the smooth muscle myosin chain promoter can direct expression to vascular tissue

Figure 3: Promoter sequence driving tissue specific gene expression

Figure 4a

CAACCACTACCTGAGCACCCAGTTCAAGAGACTGGGTGCTCAGGTAGTGGTTGTC

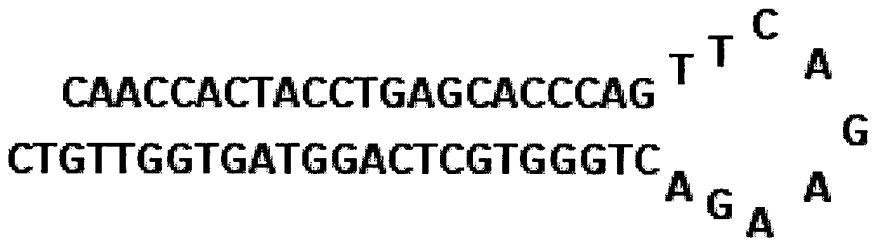


Figure 4b

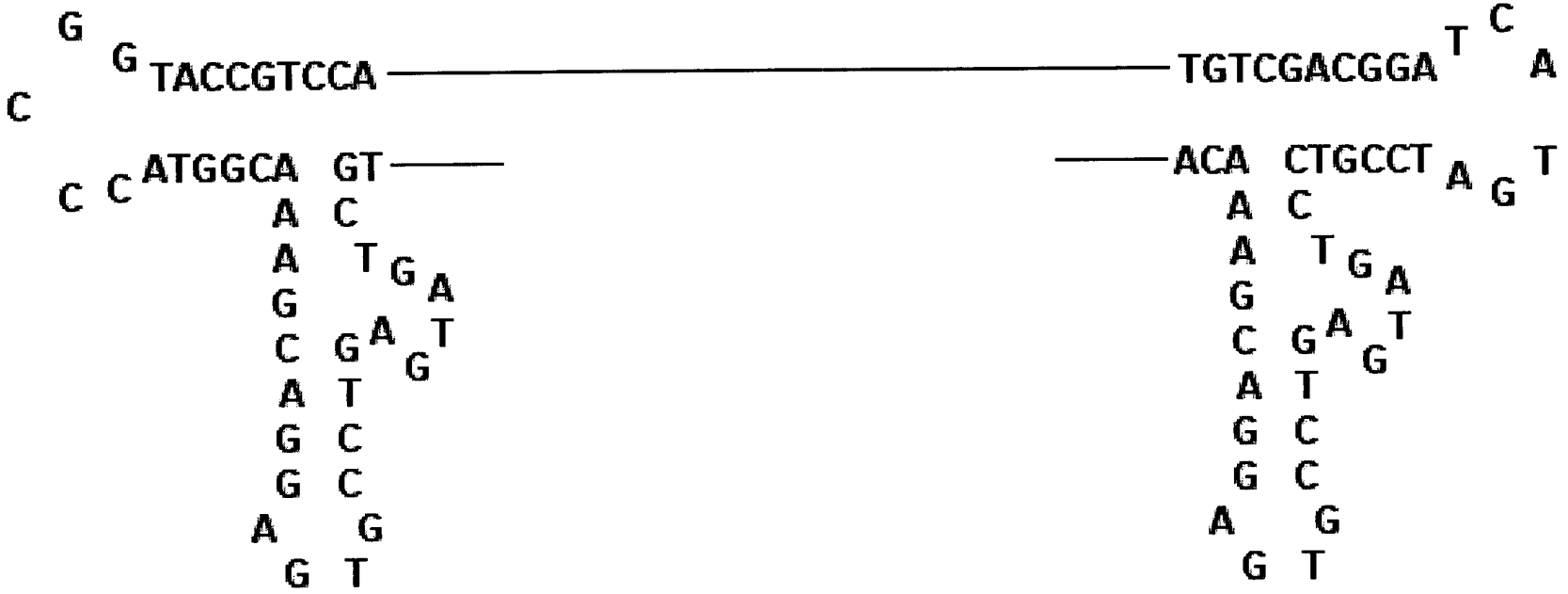


Figure 4c

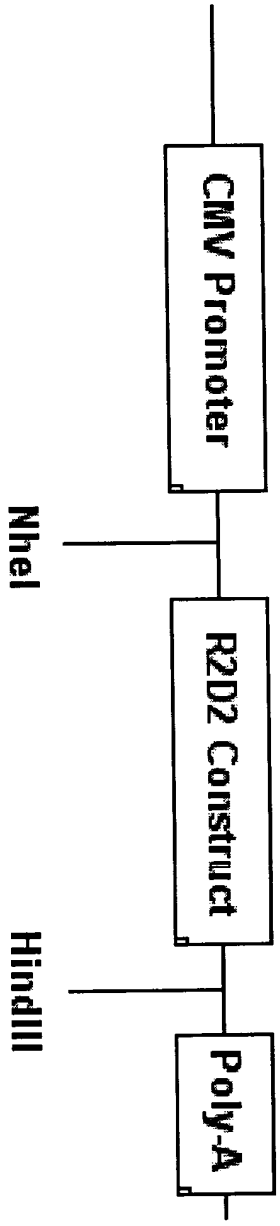


Figure 4D

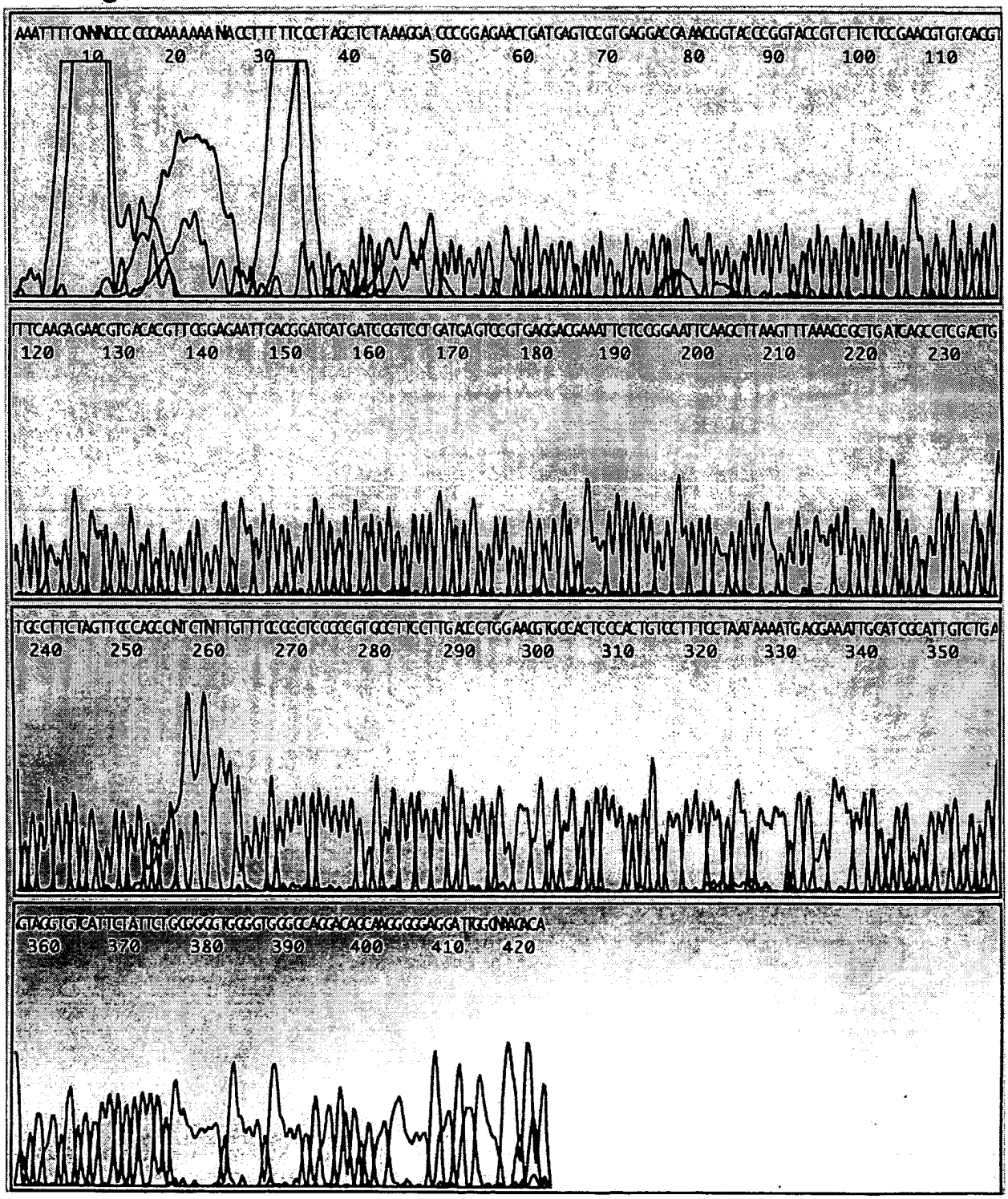

10 20 30 40 50 60 70 80 90 100 110
120 130 140 150 160 170 180 190 200 210 220 230
240 250 260 270 280 290 300 310 320 330 340 350
360 370 380 390 400 410 420

Figure 4E

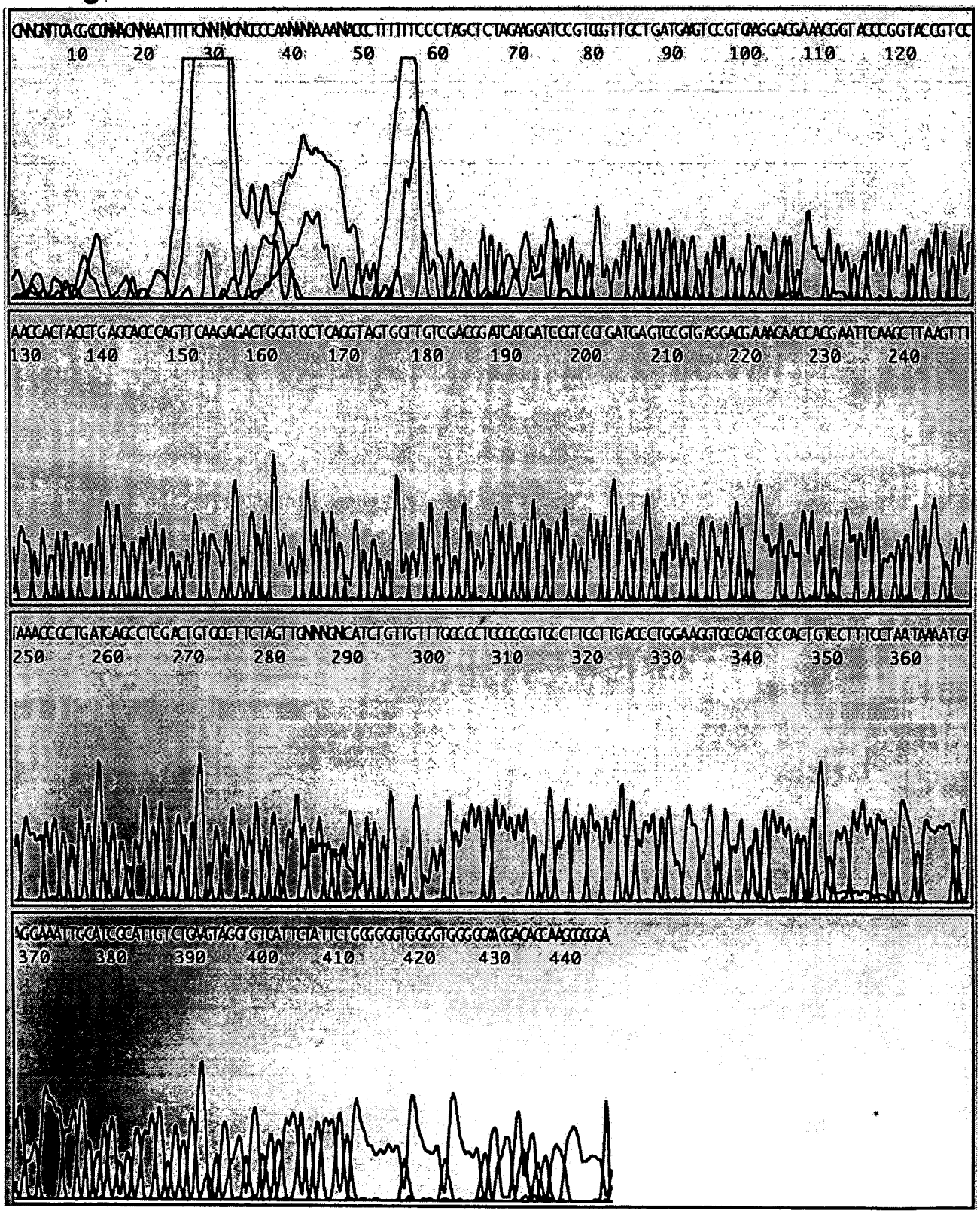

10 20 30 40 50 60 70 80 90 100 110 120
130 140 150 160 170 180 190 200 210 220 230 240
250 260 270 280 290 300 310 320 330 340 350 360
370 380 390 400 410 420 430 440

Figure 4F

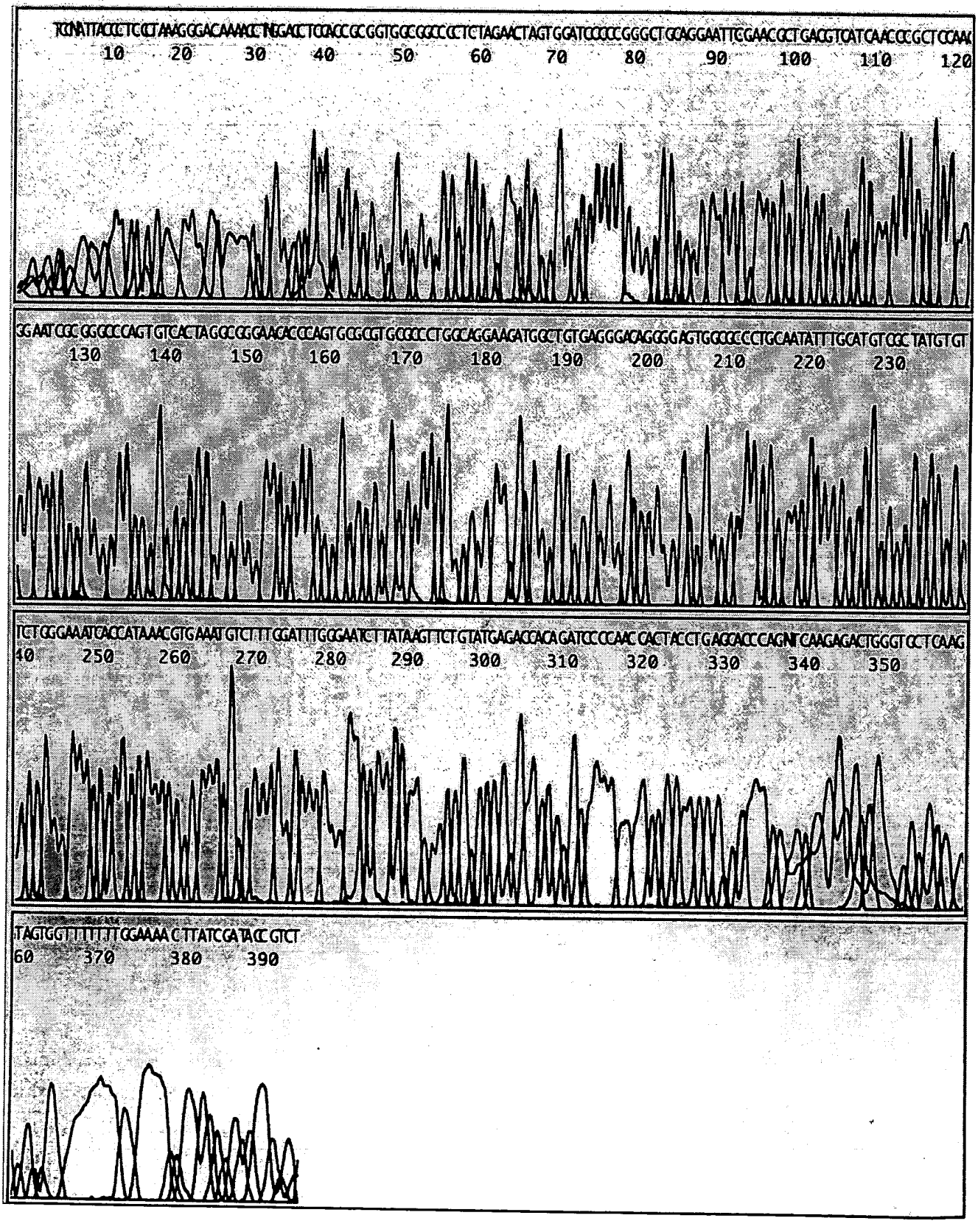

10 20 30 40 50 60 70 80 90 100 110 120
130 140 150 160 170 180 190 200 210 220 230
240 250 260 270 280 290 300 310 320 330 340 350
360 370 380 390

Figure 4G

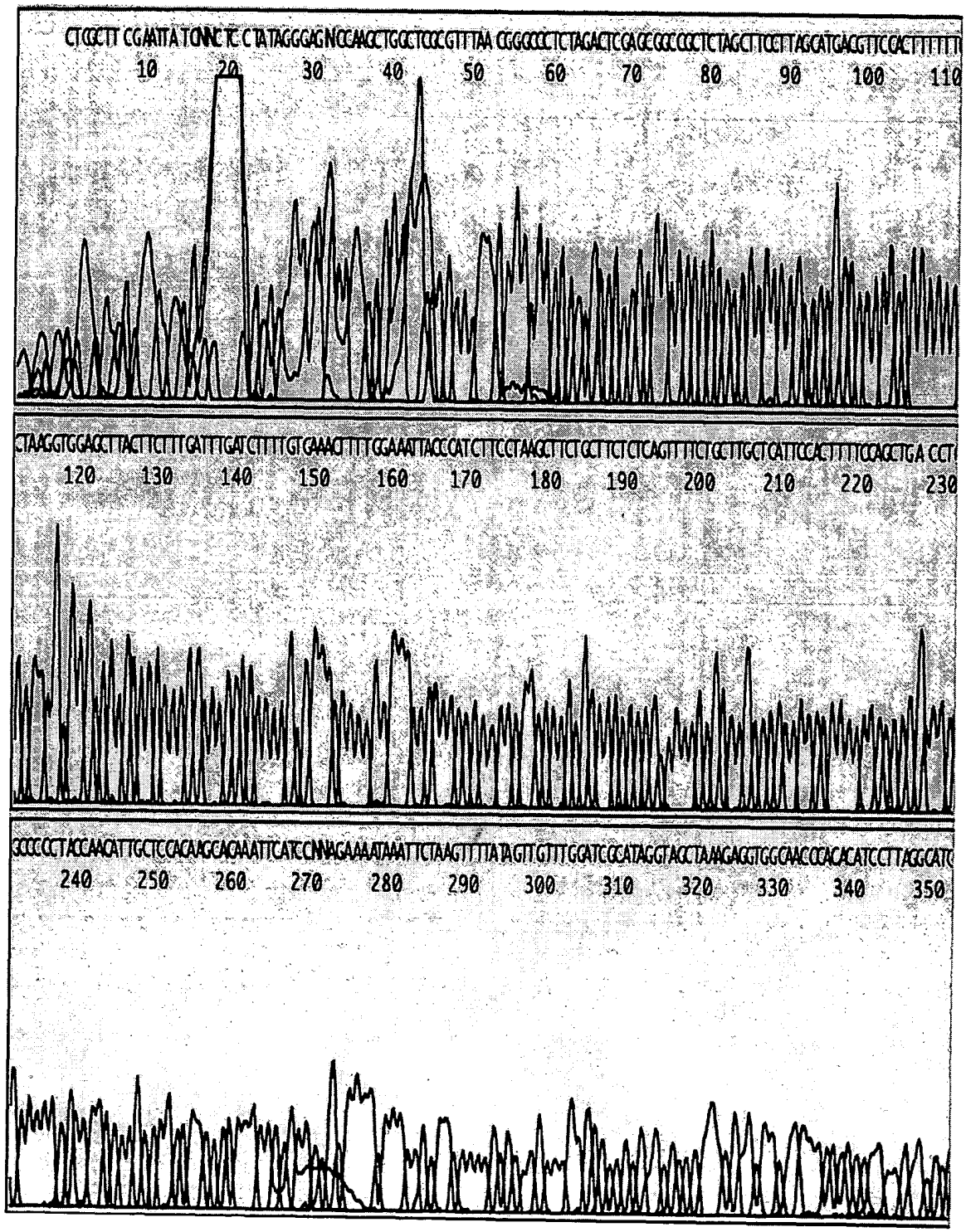

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Figure 5a

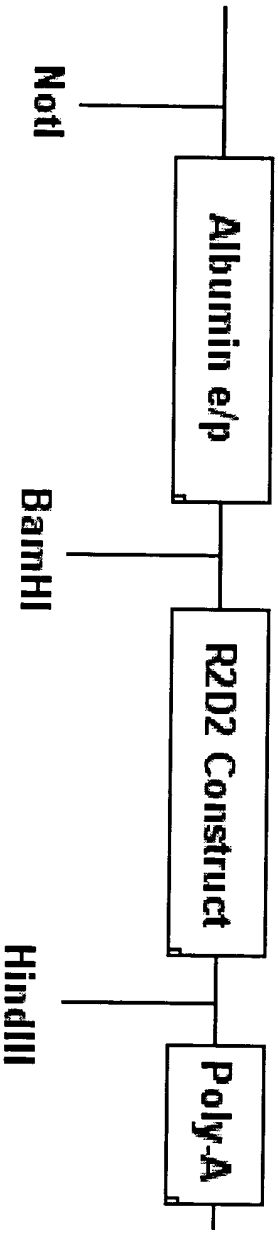


Figure 5b

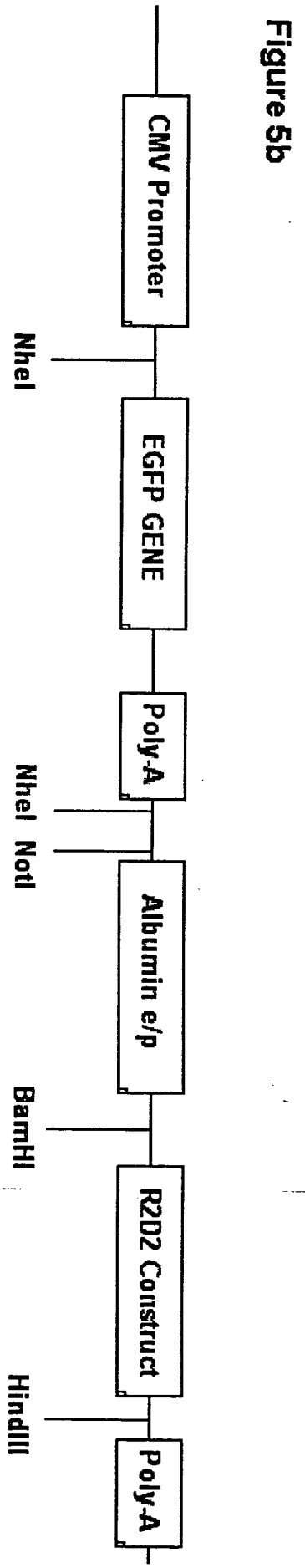

CMV Promoter
NheI
EGFP GENE
Poly-A
NheI NotI
Albumin e/p
BamHI
R2D2 Construct
HindIII
Poly-A

Figure 5c

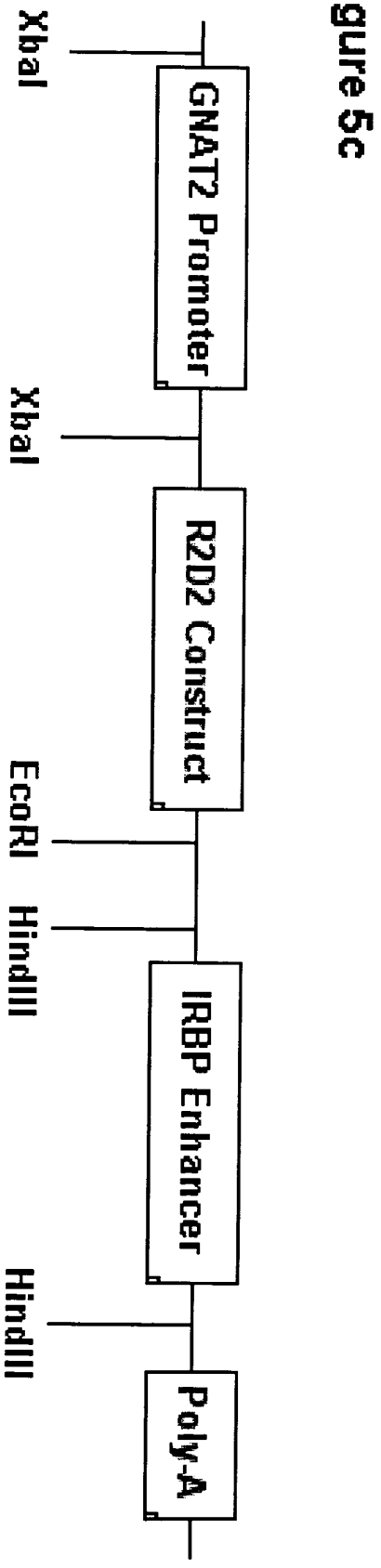

XbaI
GNAT2 Promoter
XbaI
R2D2 Construct
EcoRI
HindIII
IRBP Enhancer
HindIII
Poly-A

Figure 6

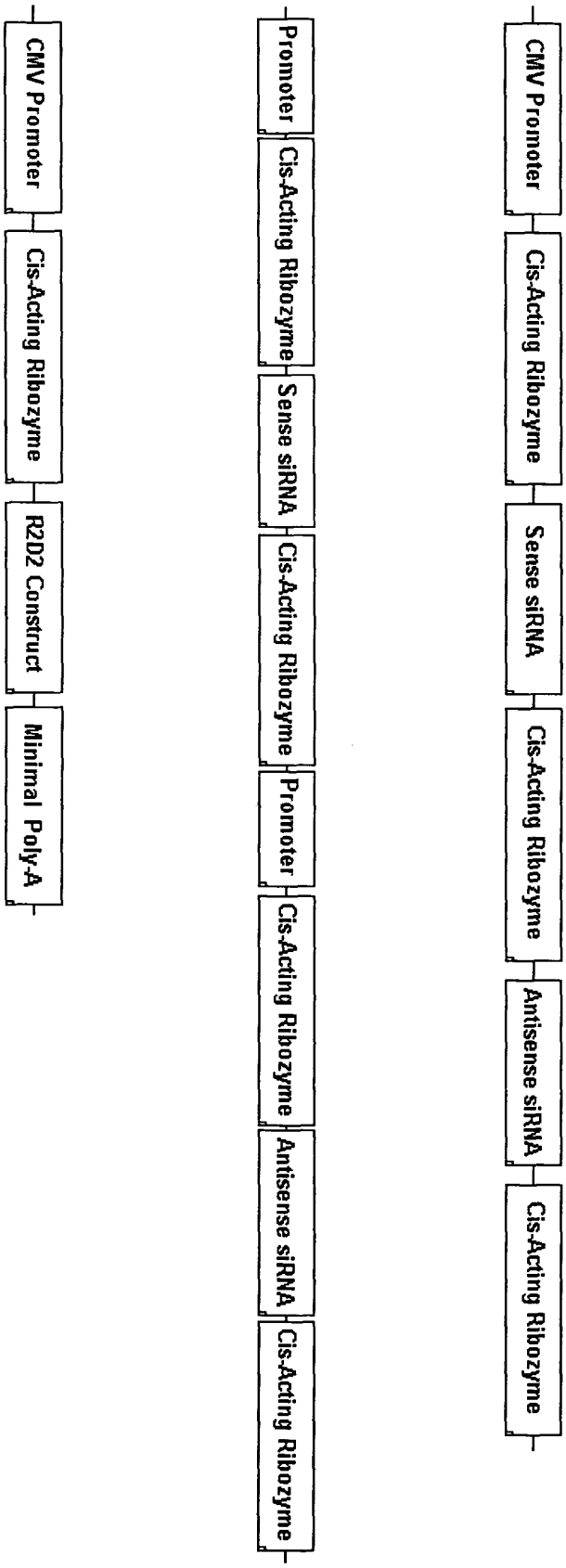
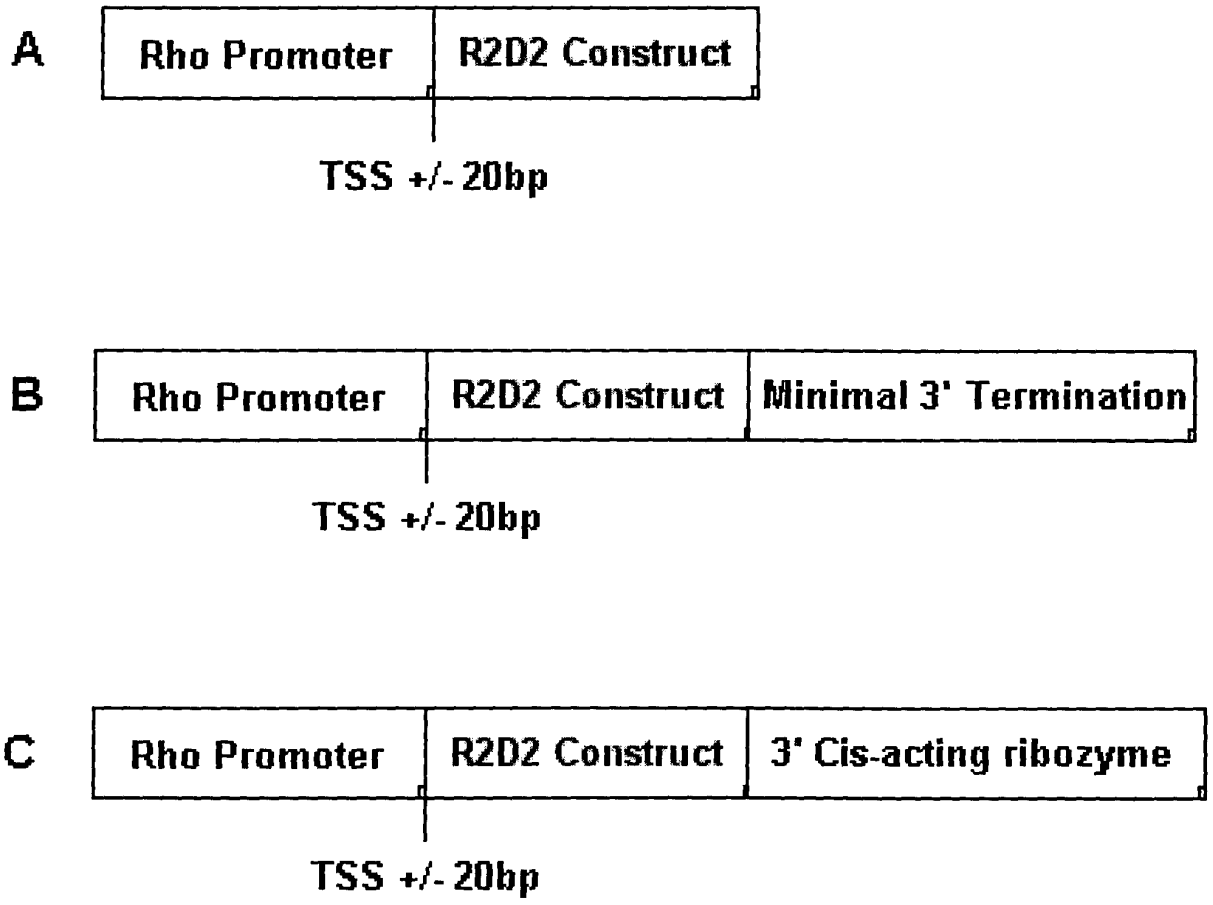

CMV Promoter
Cis-Acting Ribozyme
Sense siRNA
Cis-Acting Ribozyme
Antisense siRNA
Cis-Acting Ribozyme
Promoter
Cis-Acting Ribozyme
Sense siRNA
Cis-Acting Ribozyme
Promoter
Cis-Acting Ribozyme
Antisense siRNA
Cis-Acting Ribozyme
CMV Promoter
Cis-Acting Ribozyme
R2D2 Construct
Minimal Poly-A

Figure 7



Title. COMPOSITIONS AND METHODS FOR TISSUE SPECIFIC OR INDUCIBLE INHIBITION OF GENE EXPRESSION
Inventors: Allen et al.
Express Mail Label No. EL932952529US
Atty Docket No. MUR-005
Atty: Diana M. Steel